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Rice, tobacco, potatoes: can plants be genetically engineered to produce edible vaccines?

A SPOONFUL OF ANTIGEN

Immunisation without needles could have medical and technical advantages as well as being less traumatic for children. **Alison Tonks** reports

Any parent who has ever taken their child to a seemingly endless series of vaccinations armed with pacifiers, lollipops, and a pack of lies about how much it will hurt must have hoped that one day someone would come up with a better way to protect infants from infections. A few may even have looked on wistfully as the oral polio vaccine went down in one and wondered why all vaccines weren't that simple. Fortunately, scientists love their children too. For the past 15 years they have been looking for the best way to produce vaccines you can eat.

The original idea was simple. Genetically engineer an edible fruit or vegetable so that it contains a vaccine and feed it to children. Early pioneers started experimenting with carrots, bananas, tomatoes, soya beans, and corn. One team led by Charles Arntzen, the US based grandfather of edible vaccines, made it all the way to phase I human trials with potatoes engineered to produce harmless antigenic proteins from enterotoxigenic *Escherichia coli*, Norwalk virus, and hepatitis B virus. In a series of elegant experiments, volunteers who ate the potatoes mounted a limited immune response to all three.[1-3]

Now though, the science has moved on, and along with it the aspirations of Professor Arntzen and other enthusiasts. Edible vaccines have grown up during the past five years, and whole fruit and vegetables are off the menu. Scientists now see genetically engineered plants not as food but as an efficient production system for antigenic proteins that can be processed into pills or capsules containing fixed reproducible (and marketable) doses.

Earlier this summer, a team of scientists from Japan reported preliminary success with rice engineered to carry a vaccine against subunit B of the cholera toxin.[4] Mice fed the rice produced neutralising antibodies in their gut mucosa that seemed to protect them from an oral challenge with the cholera toxin. Professor Hiroshi Kiyono, from the division of mucosal immunology at the University of Tokyo has high hopes for rice as a vehicle for vaccines against cholera and other pathogens but concedes they have a lot more work to do before testing the vaccines in humans. Professor Arntzen and his team at Arizona State University are getting closer with an oral vaccine against Norwalk virus grown in a type of wild tobacco. "Exhaustive laboratory experiments show that this vaccine induces a powerful immune response in mice," he says. "Preliminary trials in humans should be underway within a year." Others are experimenting with tobacco containing the shiga toxin from *E coli*.[5] Plant derived vaccines against plague and anthrax are also at an early stage of development, driven by the threat of bioterrorism and funded by the research arm of the US army.

How to do it

There are essentially three ways to encourage plants to make foreign proteins from human pathogens, according to Professor Julian Ma, a leading vaccine researcher from St George's Hospital in London. You can take a gene from a pathogen such as cholera and insert it directly into the plants cells (with a little help from a common soil bacterium called *Agrobacterium*), which then produce the antigenic protein you plan to use as a vaccine. But this method, called transformation, is a bit slow and yields are low. To speed things up, you can insert the gene into a virus first, then infect the plant with it. The antigenic protein is produced quickly and efficiently during rapid viral

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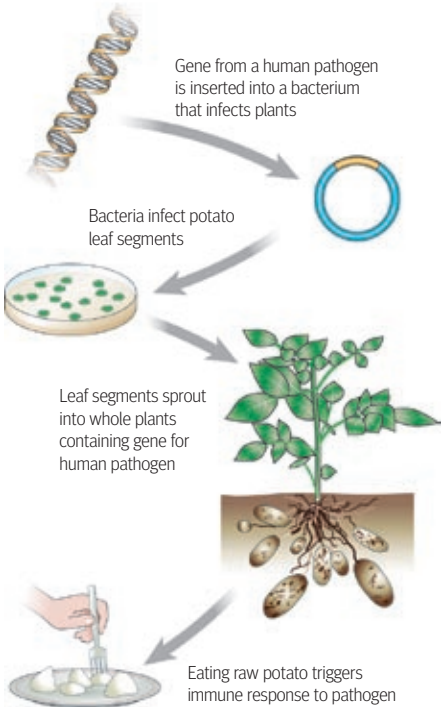
replication. This method is faster than direct transformation but carries with it the potential environmental hazard of fully infective plant viruses.

So in the most recent twist, scientists have found a way to deconstruct the viral vector, making it a harmless factory for vaccine. "The deconstructed viral vector approach is rapidly becoming the technology of choice for scientists working on these vaccines," says Professor Arntzen. "It's extremely efficient and generates more protein per kilo of plant than going down the route of creating transgenic plants." Which is just as well when you consider that in early experiments with potatoes, the concentrations of antigens in the samples were so low that volunteers had to eat at least 100 g of raw potatoes to generate an immune response.[3] The latest technique works best in a variety of wild tobacco.

Do we need plant derived vaccines?

Infectious diseases are responsible for 63% of the child deaths worldwide. Many of these deaths are preventable with the right vaccine. One in five children worldwide, or about 33 million a year, don't even get the basic vaccines such as measles.[6] So there's an urgent need for novel vaccine technologies to help reach them. Oral vaccines from genetically engineered plants have many theoretical advantages over conventional vaccines, almost all of which must be injected. They would be needle-free, making them easier and cheaper to use. Eventually, large numbers of children could be vaccinated without help from expensively trained health professionals, without the screaming, and without adding used and bloody needles to their already hazardous environment. Plant derived vaccines may even increase compliance with voluntary vaccination programmes.

Vaccines in freeze dried plants could be transported and stored at room temperature, unlike conventional vaccines, which require an unbroken chain of refrigeration from manufacture to administration. Experts estimate that it costs $200m-$300m (£98m-£148m; €145m-€218m) a year to keep a vaccine's "cold chain" intact.[4] The costs and logistics of distribution are often too much for developing nations with poor infrastructure and unreliable electricity supply. Crops




CHARLIE ARNTZEN

Pioneering: Charles Arntzen's potato pills

such as rice and tobacco are cheap to grow, relatively easy to scale-up locally, and sustainable long term.[7]

Arguably the biggest advantage, however, is that plant derived vaccines taken by mouth induce immunity at the mucosal surfaces such as the gut, the first line of defence against intestinal pathogens *Vibrio cholerae* and *E coli*.[8] Traditional vaccines given by injection induce only a systemic immune response, by which time potentially lethal infections have already broken through the mucosal defences. Mucosal vaccines, such as those being developed in rice and tobacco induce antibodies at the mucosal point of entry, as well as systemically. The World Health Organization, the US National Institutes of Health, and the Bill and Melinda Gates Foundation all believe that mucosal vaccines are a key development in the defence against pathogens that invade the body through mucosal surfaces, including HIV and influenza virus.

Getting beyond the drawing board

The technology may be feasible but someone has to pay. And so far few companies are willing to invest the $50m-$100m it would take to produce a viable plant derived vaccine.[5] Enthusiasts such as Professor Arntzen are frustrated by this lack of financial support for product development but accept that the regulatory uncertainties surrounding plant derived vaccines causes many companies to hesitate. GlaxoSmithKline and Merck spent a billion dollars each getting a recently approved vaccine against human papilloma virus to market. With sums like that at stake it's hardly surprising that big drug companies are cautious. "We also need to consider the fact that vaccines against common infectious diseases must be affordable to the developing countries that need them most, even though this limits manufacturer's profit margins," notes Professor Arntzen. "Unfortunately this makes blockbuster cancer treatments or drugs for Alzheimer's disease more financially attractive to companies in the developed world than plant derived vaccines."

Money isn't the only problem, however. Plant derived vaccines lie in a kind of no mans land between farming and pharmaceuticals. The regulatory rule book is still being written. Plants that produce antigenic proteins are regulated under the same

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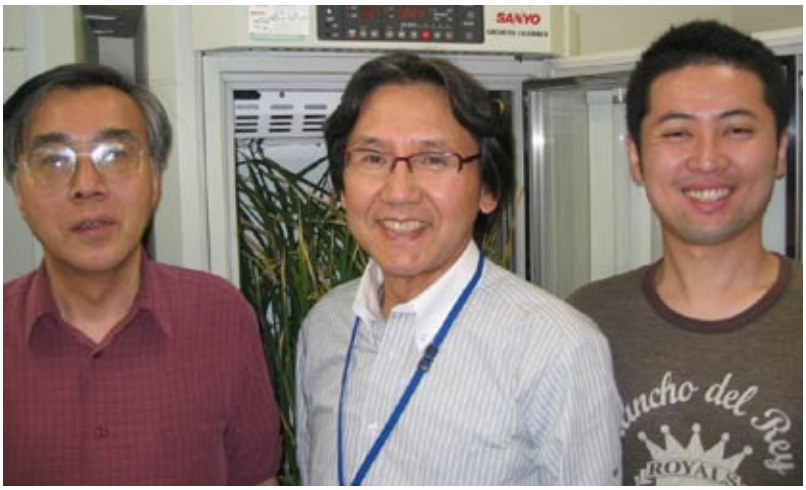
framework as genetically modified crops in many jurisdictions.[6] Large scale production is likely to attract similar social and political unease. How, for example, would producers stop their transgenic food crops contaminating other crops and getting into the food chain? A Canadian collaboration of scientists and ethicists is already studying these and other social objections to plant derived vaccines, hoping to head off the inevitable objections before they turn into outright social rejection of the technology.[9]



The Mucorice team from left: Yoshikazu Yuki, Hiroshi Kiyono and Tomonori Nochi

Some leading observers and the WHO believe all plants engineered to produce therapeutics proteins, including vaccines, should be grown in greenhouses to prevent genetic drift.[10] Professor Kiyono agrees that transgenic rice would have to be grown in hermetically sealed conditions to prevent contamination of the Japanese staple crop. The contamination issue has driven others back towards inedible crops such as tobacco: "Using tobacco avoids any concerns about contaminating the food chain. It's ironic that a Japanese team are still working with a rice based production system, when their countrymen are so concerned about the genetic purity of their food rice crops. While their science is elegant, I don't see this being accepted as the vaccine technology of the future," says Professor Arntzen.

Regulators such as the European Medicines Agency remain uneasy, even about tobacco. "Transgenic plants producing antigenic proteins have been around for 15 years, but drug companies and regulatory agencies still think of them as a prototype technology," says Professor Ma. Scientists are more likely to overcome regulatory hurdles with pills and capsules than with whole bananas, but they still have a lot of persuading to do. One strategy is to test the water with vaccines for animals first, and Dow Agrosciences have recently obliged by gaining a licence for a plant derived vaccine against Newcastle disease in chickens.[11] Researchers are also testing the regulators with more familiar agents produced in plants or plant cells such as intrinsic factor, insulin, and aprotinin to see how they react. An Israeli biotechnology company Protalix recently gained the US Food and Drug Administration's approval to start advanced human trials of their new treatment for Gaucher disease—glucocerebrocidase grown in cultures of genetically engineered carrot cells. If these and other therapeutic ventures are successful, oral vaccines grown in plants or plant cell cultures could be next.

But there's one more hurdle to jump first—the theoretical possibility that oral mucosal vaccines might induce tolerance rather than immunity. Tolerance is a mechanism by which an orally delivered antigen might somehow disable or at least interfere with the systemic immune system. The result could be a disastrous "antivaccine" that told the body to ignore invading pathogens and not respond to infection. It may be the reason we don't mount an immune response to food.

"Tolerance is only a theoretical possibility, but as yet no one quite understands how to overcome it," says Professor Ma, although there's a scientific consensus that in reality, tolerance is highly unlikely to happen. We can't learn anything from the oral polio vaccine, which is a systemic not a mucosal vaccine.

"I sensed a little despondency initially at our recent scientific meeting in Verona" he says, "but vaccine development has always been a long slow process. The new vaccine against human papilloma viruses was in the pipeline for at least 23 years. We've only been going for 15, and our technology is still fairly young. If it was that simple, we'd have got there years ago. There's still plenty to look forward to, and the pace of development continues to accelerate."

Professor Arntzen's oral vaccine against Norwalk virus, which is rapidly approaching human testing, seems to be the plant derived vaccine most likely to cross the finish line first. Who knows how long it will take, but an oral vaccine against such a high impact pathogen would be a fitting tribute to the man who first thought it might be possible to grow oral vaccines in plants.

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